should have spots of corresponding R_f values.

[39 FR 34032, Sept. 23, 1974; 48 FR 11427, Mar. 18, 1983, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.312 Atomic absorption method for determining the zinc content of zinc bacitracin.

(a) *Equipment.* An atomic absorbance spectrophotometer equipped with a zinc hollow-cathode discharge lamp, an air-acetylene flame, a nebulizer-burner system for introducing the sample solution into the flame, an optical dispersing device (such as a monochromator) for isolating a resonance line of zinc from others produced by the emission source, and a suitable radiation detector and recorder.

(b) *Preparation of working standard and sample solutions*—(1) *Workingstandard solutions.* Prepare a standard stock solution containing 10 milligrams of zinc per milliliter as follows: Weigh 3.11 grams of zinc oxide into a 250-milliliter volumetric flask, add 80 milliliters of 1*N* HCl, warm to dissolve, cool to room temperature, and dilute to volume with water. Dilute aliquots of this standard stock solution with 0.001*N* HCl to obtain three working standard solutions containing respectively 0.5, 1.5, and 2.5 micrograms of zinc per milliliter.

(2) *Sample solution.* Accurately weigh approximately 200 milligrams of the sample into a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.01*N* HCl. Transfer a 2.0-milliliter aliquot of this solution to a 200-milliliter volumetric flask and dilute to volume with 0.001*N* HCl.

(c) *Procedure.* Using 0.001*N* HCl as the blank, adjust the absorbance of the instrument to zero at a detection wavelength of 213.8 nanometers. Determine the absorbance of each standard solution and the sample solution at 213.8 nanometers.

(d) *Calculations.* Plot the absorbance versus the concentration of each of the working standard solutions. Draw a straight response line of best fit through these points. Read the concentration of zinc in micrograms per milliliter corresponding to the absorbance of the sample solution. Calculate the percent zinc in the sample as follows:

$$\text{Percent zinc} = \frac{C \times 100{,}000}{\text{Milligrams of sample} \times (100 - m)}$$

where:

C=Concentration of zinc in the sample solution in micrograms per milliliter;

m=Percent moisture in the sample.

[40 FR 15088, Apr. 4, 1975]

§ 436.316 Determination of penicillin G content.

(a) *Reagents.* The reagents are freshly prepared every three days and are of such quality that when used in this procedure with an authentic sample of penicillin G, not less than 97 percent of penicillin G is recovered.

(1) *Amyl acetate (iso-amyl acetate) solution.* Saturate the amyl acetate (boiling range 138.5° C—141.5° C) with the *N*-ethylpiperidine salt of penicillin G by adding 2 milligrams of the salt for each 1.0 milliliter of the solvent. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(2) *Acetone solution.* Saturate reagent grade acetone with the *N*-ethylpiperidine salt of penicillin G using 3 milligrams of salt for each 1 milliliter of acetone. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(3) *N-ethylpiperidine solution. N*-ethylpiperidine (boiling range 129.5° C—131.0° C) should be stored in brown bottles in a refrigerator. Dilute 1.0 milliliter of this reagent with 4.0 milliliters of amyl acetate. Saturate this solution with the *N*-ethylpiperidine salt of penicillin G, using about 3 milligrams of the salt for each 1.0 milliliter of solution. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(4) *Phosphoric acid solution.* Prepare by dissolving 1.0 milliliter of reagent grade phosphoric acid (85 percent) in 4.0 milliliters of water. Cool to 0° C—8° C and shake before using.

(5) *Silica gel.* Use dry silica gel (mesh size 6–16, Tyler standard). Place about

0.5 gram of the silica gel in a micro filter funnel (approximately 10-millimeter diameter) having a fritted-glass disc of medium porosity.

(b) *Procedure.* Accurately weigh from 60 to 70 milligrams of the sample to be tested, except if penicillin G procaine is to be tested weigh 90 to 100 milligrams of sample, into a glass test tube or glass vial of approximately 10–milliliter capacity. Add 2.0 milliliters of water to dissolve or suspend (procaine) the penicillin and cool to 0° C—5° C. Add 2.0 milliliters of amyl acetate solution and 0.5 milliliter of phosphoric acid solution, stopper and shake the container vigorously for approximately 15 seconds. For penicillin G procaine, add a second 0.5-milliliter portion of phosphoric acid solution and shake vigorously. Centrifuge to obtain a clear separation of the two layers (approximately 20 seconds). If any penicillin procaine remains undissolved, add a third 0.5-milliliter portion of phosphoric acid solution, shake the container vigorously, and centrifuge. After centrifuging, remove as much of the amyl acetate layer as possible, usually about 1.7 milliliters to 1.8 milliliters, with a suitable hypodermic needle and syringe and place the portion removed into the filter funnel containing silica gel, described in paragraph (a)(5) of this section. Allow the amyl acetate to remain in contact with the silica gel for exactly 20 seconds, then apply suction and collect the filtrate in a small test tube placed in a suction flash surrounded by cracked ice. Pipet a 1.0-milliliter aliquot of the amyl acetate filtrate into a tared flat-bottom glass tube (approximately 15 x 50 millimeters) containing 1.0 milliliter of acetone solution and 0.5 milliliter of *N*-ethylpiperidine solution. The time elapsing between acidification and the addition of the filtrate to the above reagents should not be more than 3 minutes. Place the glass tube containing the mixture into a large weighing bottle, stopper the bottle and allow to stand for not less than 2 hours in a refrigerator at 0° C—8° C. Remove the liquid from the precipitate by means of a tared micro filter stick and wash with a total of 1.0 milliliter of acetone solution adding the latter by means of a hypodermic syringe equipped with a fine needle. Place the filter stick inside the glass tube, dry under vacuum at room temperature for not less than 1 hour, and weigh. (The *N*-ethylpiperidine penicillin G residues can be saved for saturating reagents).

(c) *Calculations.* Calculate the percent penicillin G content as follows:

$$\text{Percent penicillin G content} = \frac{\text{Milligrams } N\text{-ethylpiperidine penicillin precipitate} \times 149.4}{\text{Weight of sample in milligrams}}$$

[42 FR 59857, Nov. 22, 1977]

§ 436.317 Solubility characteristic test for griseofulvin (ultramicrosize) tablets.

(a) *Apparatus*—(1) *Vessel.* A cylindrical glass tank. The approximate dimensions are 40 centimeters in diameter and at least 23 centimeters in height.

(2) *Heating system.* A 1,500-watt immersion heating element connected to a partial immersion, contact thermometer and an appropriate control relay.

(3) *Circulating system components.* The circulating system consists of three different circulating devices:

(i) Circulating pump of a centrifugal, immersion type. Tubing approximately 1 centimeter outside diameter and 46 centimeters in length is attached to the pump outlet producing a flow rate of approximately 1,600 milliliters per minute when operated as described.

(ii) A ''4-element stirrer'' consisting of a motor and a shaft approximately 45 centimeters long and 8 millimeters in diameter. The motor rotates the vertical shaft in a clockwise direction at approximately 180 revolutions per minute. There are 4 elements or sets of stirring blades on the shaft. One set, located at the bottom of the shaft, is a 3-bladed element of 2.5 centimeters